FACILE SYNTHESIS OF (2-BENZIMIDAZOLYL)-1-AZAAZULENES, (2-BENZOTHIAZOLYL)-1-AZAAZULENES, AND RELATED COMPOUNDS AND EVALUATION OF THEIR ANTICANCER IN VITRO ACTIVITY†

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Abstract – Facile syntheses of 2-, 3-, and 8-(2-benzimidazolyl)-1-azaazulenes (2a–c, 5, 7, 9) and 2-, 3-, and 8-(2-benzothiazolyl)-1-azaazulenes (13b–c, 16, 17, 18) were achieved by the condensation of corresponding 1-azaazulene-carbaldehydes with o-phenylenediamine and 2-aminothiophenol in alcoholic solvents at rt or under reflux under airobic conditions. Reaction of 1-azaazulene-carbaldehyds with 2-aminophenol gave Schiff’s bases (10a–c, 11, 12). Reaction of 2-chloro-1-azaazulene-3-carbaldehyde (1a) with 2-aminothiophenol in refluxing 1-BuOH gave benzothiazapine-fused 1-azaazulene (20). Reaction of 4-amino-3-mercapto-4H-1,2,4-triazoles (21a–d) with in refluxing 1-BuOH gave triazolothiadiazapine-fused 1-azaazulene (22a–d). The structure of trifluoromethyl derivative (22c) was determined by X-ray structure analysis. 3-(2-Benzimidazolyl)-2-chloro-1-azaazulene (2a) showed anticancer activity against HeLa S3 cells (IC50: 5.3 µM).

† Dedicated to Professor Dr. Ryoji Noyori on occasion of his 70th birthday.
INTRODUCTION

Benzazoles such as benzimidazoles, benzothiazoles, and benzoazoles, are important subunits for the development of functional molecules of pharmaceutical and biological interest. Substituted benzothiazoles and benzimidazoles have found application in a wide range of therapeutic areas such as antiulcers, anticancers, antihistamies, antifungals, and antivirals to name a few.\textsuperscript{1-10}

The chemistry of azaazulenes\textsuperscript{11} is of interest for their physiological properties\textsuperscript{12,13} as well as physical and chemical properties. Therefore, it is expected that benzazoyl-1-azaazulenes have potential bioactivities. Numerical methods for the synthesis of benzazoles are reported. We previously reported that 2-chloro-1-azaazulene-3-carboaldehyde reacted with \(\text{o-phenylenediamine}\) to give 3-(benzimidazol-2-yl)-2-chloro-1-azaazulene.\textsuperscript{14} Therefore, we expand the investigation about the syntheses of benzazoyl-1-azaazulenes by condensation reaction of formyl-1-azaazulenes with \(\text{o-phenylenediamine}\), 2-aminophenol, and 2-aminothiophenol.

RESULTS AND DISCUSSION

Synthesis of (benzimidazol-2-yl)- and (benzothiazol-2-yl)-1-azaazulenes

Under open air conditions, the reaction of 2-chloro-1-azaazulene-3-carbaldehyde (1a) with \(\text{o-phenylenediamine}\) in EtOH for 48 h at rt underwent to give 2a in 78\% yield along with recovered (1a : 15\%). In the reaction, the intermediate imine (3a) was not observed. Reactivity of 1b was slightly low than that of 1a, and heating under reflux of 1b with \(\text{o-phenylenediamine}\) in EtOH for 37 h gave 2b in 59\% yield along with a trace amount of 1b, and 3b was not obtained. Reactivity of 1c was moreover low, and when 1c was treated with \(\text{o-phenylenediamine}\) in EtOH for 44 h at rt to give the imine derivative (3c) in 62\% yield along with 1c (15\%). Heating under reflux of 1c with \(\text{o-phenylenediamine}\) in 1-BuOH for 37 h achieved 2c in 88\% yield. Extent of electron-donation of the sunstituent at C-2, which
conjugated with carbonyl at C3, would affect the reactivity.
Similar treatment of 3-bromo-1-azaazulene-2-carbaldehyde (6) and 1-azaazulene-8-carbaldehyde (8a) with o-phenylenediamine in EtOH at rt gave 7 and 9 in 40% and 38% yields, respectively. In these reactions, 6 and 8 were not recovered because of their lability.
The structures of obtained compounds were determined by spectroscopic data as well as elemental analyses as shown in EXPERIMENTAL. The imine (3c) was decided by X-Ray structure analysis and its ORTEP drawing is shown in Figure 1.

Figure 1. ORTEP drawing with thermal ellipsoids (50% probability) of 3c.

Next, we examined the reaction of 1-azaazulene-3-carbaldehydes (1a-1c) with 2-aminophenol. When 1a was treated with 2-aminophenol in refluxing EtOH for 15 min, 10a precipitated as orange crystals in 60% yield. When the heating was continued, the precipitates were dissolved and after heating of the mixture for 15 h, compound (11) was obtained in 37% yield. When the reaction was carried out at 50 °C for 40 min, 10a was obtained in 80% yield. Similarly, treatment of 1b with 2-aminophenol in

10a : X = Cl  
10b : X = OEt  
10c : X = NH₂ 

11

12
refluxing EtOH for 24 h and treatment of 1c with 2-aminophenol in refluxing 1-BuOH for 22 h gave 10b (48%) and 1c (91%), respectively. Similar treatment of 4 with 2-aminophenol in refluxing EtOH gave 12 in 54% yield.

It is known that the reaction under presence of oxidizing reagents facilitates the cyclization to benzimidazoles and benzoxazoles.\textsuperscript{16-18} Therefore, we performed the reaction of 1b with 2-aminophenol in the presence of DDQ in EtOH under reflux for 24 h. In the reaction, a new spot was seen on the TLC, but after work-up, 1b (69%) was recovered with a trace amount of 2b, and benzoxazole derivative was not obtained. It seems that 1b formed CT-complex with DDQ, and the reaction underwent scarcely. Then, we treated 1c with 2-aminophenol under reflux for 48 h in the presence of I\textsubscript{2}, but a complex mixture was produced and the benzoxazole derivative was not obtained again.

Next, we examined the synthesis of benzothiazole derivatives. It is expected that high nucleophilicity of S-atom would facilitate the attack to imine moiety. Indeed, the reaction of 1b with 2-aminothiophenol in EtOH under reflux for 4.5 h underwent and 13b was obtained in 66% yield. Similar treatment of 4, 6, and 8b with 2-aminothiophenol gave 16 (53%), 17 (32%), and 18b (54%), respectively.

Interestingly, when 1c was treated with 2-aminothiophenol in EtOH under reflux, the reaction was not proceeded. But when 1c was treated with 2-aminothiophenol in 1-BuOH under reflux for 190 h, 13c (36%) and 2-amino-1-azaazulene (14: 59%) were obtained. In the reaction, benzothiazole was detected.

Plausible reaction mechanism is shown in Scheme 1. At first, the imine (A) would be produced, and a successive cyclization affords B. Auto oxidation of B furnishes 13c (path a), and elimination of benzothiazole from B gave 14 (path b).

\begin{center}
\begin{tabular}{l}
13b : X = OEt \\
13c : X = NH\textsubscript{2} \\
14 \\
15 \\
16 \\
17 \\
18
\end{tabular}
\end{center}

Above consideration suggested that the use of oxidation reagent would improve the reaction. Therefore,
we carried out the reaction of 1c with 2-aminothiophenol in the presence of I2 in 1-BuOH under reflux for 48 h, and 13c (24%) and 15 (52%) were obtained together with 1c (7%). Attack of S-atom to aldehyde group of 1c and successive oxidation by I2 would produce 15. When the reaction was carried out in DMF at 100 °C for 24 h, 13c was obtained only in 25% yield along with 1c (51%). In this case, the compound (15) was not obtained. Reaction of 1c with 2-aminothiophenol in the presence of FeCl3 in 1-BuOH under reflux for 48 h gave 13c in 31% along with 1c (11%).

The structure of 13c was confirmed by X-ray structure analysis (Figure 2). The bond length of C(2)-N(amino) is rather short (1.331 Å), and hydrogen bonding N-H---N (2.08 Å) is observed. The results suggest that contribution of resonance form (13c-A) is large. The electronic spectra of 13b, 13c,

![Scheme 1](image)

**Scheme 1**

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![Figure 2](image)

**Figure 2.** ORTEP drawing with thermal ellipsoids (50% probability) and selective bond lengths of 13c.
and 16 in EtOH are shown in Figure 3. Interestingly, the spectral features of 13c and 16 resemble, and those of 13c and 13b are rather different. The results suggest that tautomerisation between 13c and 19 exists in the solution.

![Figure 3](image)

Figure 3. UV and Visible spectra of 13b, 13c, and 16.

**Syntheses of benzothiazepine-fused and triazolothiadiazepine fused 1-azaazulenes**

Interestingly, the reaction of 2-chloro-1-azaazulene-3-carboaldehyde (1a) with 2-aminothiophenol showed a different aspect. Treatment of 1a with 2-aminothiophenol in EtOH under reflux gave a complex mixture and distinct product was not obtained. When the reaction performed in 1-BuOH under reflux for 45 h, cyclized compound (20) was obtained in a 41% yield. The structure of 20 was determined by spectroscopic data as well as elemental analysis. In its $^1$H NMR spectrum, low field resonated 1H singlet assigned to H-12 was observed at $\delta$ 10.61, owing to the anisotropic effect of seven-membered ring.

We expand the examination for synthesis of fused 1-azaazulenes containing thiadiazepine ring. Thus,
1a was treated with 4-amino-3-mercapto-1,2,4-triazoles (21a-d) in refluxing 1-BuOH for a few min, and 22a-d were obtained in 49%, 67%, 33%, and 43% yields, respectively. The structure of 22c was confirmed by X-ray structure analysis (Figure 4).

Figure 4. ORTEP drawing with thermal ellipsoids (50% probability) of 22c.

Biological evaluation

Some new synthesized products (2a, 2b, 2c, 5, 13b, 16) were evaluated for their anticancer activity (cytotoxic activity) against HeLa S3 cells. The IC$_{50}$ values [µM] are summarized in Table 1. In several cases (denoted >), the minimum inhibitory concentration could not be determined due to limited solubility of the compounds in the testing medium. The data revealed that compounds (2a) showed moderate activity against HeLa S3 cells, and others would be inactive (IC$_{50}$ > 30 µM).

Table 1. Cytotoxic evaluation of compounds (2a, 2b, 2c, 5, 13b, 16) expressed in µM.

<table>
<thead>
<tr>
<th></th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>5</th>
<th>13b</th>
<th>16</th>
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<tbody>
<tr>
<td>IC$_{50}$</td>
<td>5.3±0.4</td>
<td>62±3</td>
<td>&gt;9.6</td>
<td>&gt;38</td>
<td>&gt;8.1</td>
<td>&gt;4.4</td>
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CONCLUSION

Benzimidzolyl- and benzthiazolyl-1-azaazulenes are easily synthesized by the treatment of
formyl-1-azaazulenes with o-phenylenediamine and 2-aminothiophenol in alcoholic solvent under airobic conditions. Reaction of formyl-1-azaazulenes with 2-aminophenol gave only Schiff’s bases and benzoxazolyl-1-azaazulenes were not obtained. Reactions of 2-chloro-1-azaazulene-3-carbaldehyde with 2-aminothiophenol and 4-amino-3-mercapto-1,2,4-triazoles gave 5-thia-6,13-diazacyclohepta-[a]benz[f]azulene and 4-thia-2,3,5,12,12a-pentaazaazuleno[5,6-b]azulenes. Benzimidazolyl- and benzthiazolyl-1-azaazulenes showed anticancer activity (cytotoxic activity) against HeLa S3 cells.

**EXPERIMENTAL**

Mps are measured using a Yanagimoto micro-melting apparatus and uncorrected. 1H NMR spectra (including HH-COSY and CH-COSY NMR)) were recorded on a Bruker AVANCE 400S (400 MHz) and 13C NMR spectra were recorded on a Bruker AVANCE 400S (100.6 MHz) using DMSO-d6 as a solvent with TMS as an internal standard unless otherwise stated; J values are recorded in Hz. IR spectra were recorded for KBr pellets on a Nicolet FT-IR Avatar 370DTGS. Electronic spectra were recorded with Shimadzu UV-1600PC spectrophotometer using EtOH as a solvent. Elemental analyses were taken with a Perkin Elmer 2400II. Kieselgel 60 was used for column chromatography and Kieselgel 60G was used for thin-layer chromatography.

**Reactions of 2-chloro-1-azaazulene-3-carbaldehyde (1a) with o-phenylenediamine**

A mixture of 1a (0.071 g, 0.37 mmol) and o-phenylenediamine (0.042 g, 0.39 mmol) in EtOH (20 mL) was stirred for 48 h at rt. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 2a (0.081 g, 78%) and recovered (1a) (0.011 g, 15%).

2a: Red plates (from CH2Cl2-hexane), mp 221-223 °C; 1H NMR δ 12.59 (1H, s), 9.68 (1H, d, J 10.0), 8.79 (1H, d, J 9.6), 8.27 (1H, dd, J 10.0, 9.6), 8.13 (1H, dd, J 10.0, 9.6), 8.11 (1H, dd, J 10.0, 9.6), 7.76-7.67 (2H, m), 7.31-7.23 (2H, m); 13C NMR δ 155.4, 153.4, 144.9, 143.9, 141.0, 138.7 (br), 137.5, 137.2, 132.5, 132.4, 122.1, 115.1 (br), 112.1; ν max / cm⁻¹ 3368 (NH); λ max nm (log ε) 228 (4.45), 262 (4.49), 306 (4.53), 366 (3.91), 478 (3.09). Anal. Calcd for C16H10N3Cl: C, 68.70; H, 3.60; N, 15.02. Found: C, 68.57; H, 3.78; N, 14.78.

**Reactions of 2-ethoxy-1-azaazulene-3-carbaldehyde (1b) with o-phenylenediamine**

A mixture of 1b (0.061 g, 0.31 mmol) and o-phenylenediamine (0.042 g, 0.39 mmol) in EtOH (20 mL) was stirred under reflux for 37 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 2b (0.052 g, 59%).

2b: Orange needles (from CH2Cl2-hexane), mp 173-175 °C; 1H NMR δ 11.96 (1H, br s), 9.80 (1H, d, J 9.6), 8.44-8.36 (1H, m), 7.98-7.82 (3H, m), 7.72-7.64 (2H, m), 7.25-7.19 (2H, m), 4.89 (2H, q, J 7.0), 1.59 (3H, t, J 7.0); 13C NMR δ 171.6, 155.6, 146.4, 143.8, 138.5 (br), 135.6, 133.1, 132.1, 131.9, 131.8,
Reactions of 2-amino-1-azaazulene-3-carbaldehyde (1c) with o-phenylenediamine

\( a \) A mixture of 1c (0.053 g, 0.30 mmol) and o-phenylenediamine (0.099 g, 0.91 mmol) in 1-BuOH (10 mL) was heated at 70 °C for 44 h. The solvent was evaporated, and the chromatography of the residue with AcOEt gave 2-amino-3-[(2-aminophenyl)iminomethyl]-1-azaazulene (3c) (0.050 g, 62%) and recovered (1c) (0.012 g, 22%).

\( b \) A mixture of 1c (0.051 g, 0.30 mmol) and o-phenylenediamine (0.098 g, 0.90 mmol) in 1-BuOH (10 mL) was heated under reflux for 48 h. The solvent was evaporated, and the chromatography of the residue with AcOEt gave 2-amino-3-(2-benzimidazoyl)-1-azaazulene (2c) (0.068 g, 88%).

2c: Orange needles (from EtOH), mp 289-291 °C; 1H NMR \( \delta \) 12.50 (1H, s), 8.54 (1H, d, \( J = 10.4 \)), 8.20 (2H, br s), 7.92 (1H, d, \( J = 9.6 \)), 7.70-7.55 (4H, m), 7.47 (1H, dd, \( J = 10.0, 9.6 \)), 7.22 (1H, dd, \( J = 8.8, 0.8 \)); 13C NMR \( \delta \) 168.7, 160.3, 148.2, 142.7 (br), 142.4, 134.3 (br), 132.0, 130.8, 130.3, 126.3, 125.4, 121.7, 117.7 (br), 111.1 (br), 98.5; \( \nu_{\text{max}} \) / cm\(^{-1}\) 3432, 3288, 3222 (NH); \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)) 244 (4.37), 270 (4.52), 328 (4.69), 448 (3.99). Anal. Calcd for C\(_{16}\)H\(_{12}\)N\(_4\): C, 73.83; H, 4.65; N, 21.52. Found: C, 73.83; H, 4.79; N, 21.08.

3c: Red plates (from CH\(_2\)Cl\(_2\)-hexane), mp 210-212 °C; 1H NMR \( \delta \) 12.50 (1H, s), 8.54 (1H, d, \( J = 10.4 \)), 8.20 (2H, br s), 7.92 (1H, d, \( J = 9.6 \)), 7.70-7.55 (4H, m), 7.47 (1H, dd, \( J = 10.0, 9.6 \)), 7.22 (1H, dd, \( J = 8.8, 0.8 \)); 13C NMR \( \delta \) 168.7, 160.3, 148.2, 142.7 (br), 142.4, 134.3 (br), 132.0, 130.8, 130.3, 126.3, 125.4, 121.7, 117.7 (br), 111.1 (br), 98.5; \( \nu_{\text{max}} \) / cm\(^{-1}\) 3432, 3288, 3222 (NH); \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)) 226(4.39), 271 (4.30, sh), 300 (4.43), 352 (4.29), 463 (4.10). Anal. Calcd for C\(_{16}\)H\(_{14}\)N\(_4\): C, 73.26; H, 5.38; N, 20.96. Found: C, 73.56; H, 5.48; N, 20.96.

Reactions of 2-oxo-1,2-dihydro-1-azaazulene-3-carbaldehyde (4) with o-phenylenediamine

A mixture of 4 (0.0526 g, 0.30 mmol) and o-phenylenediamine (0.0339 g, 0.31 mmol) in EtOH (10 mL) was heated under reflux for 20 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 3-(2-benzimidazoyl)-1-azaazulen-2(1\( H \))-one (5) (0.0345 g, 43%).

5: Red powders (from CH\(_2\)Cl\(_2\)-hexane), mp 277-279 °C; 1H NMR \( \delta \) 12.28 (1H, s), 12.19 (1H, s), 9.38 (1H, d, \( J = 11.2 \)), 7.76-7.66 (2H, m), 7.63 (1H, dd, \( J = 11.2, 9.6 \)), 7.53 (1H, dd, \( J = 10.0, 9.2 \)), 7.43 (1H, d, \( J = 9.2 \)), 7.31 (1H, dd, \( J = 10.0, 9.6 \)), 7.22-7.13 (2H, m); 13C NMR \( \delta \) 168.1, 147.3, 145.6, 143.3 (br), 141.1, 134.5, 134.1, 133.3 (br), 131.2, 129.8, 121.5, 117.8 (br), 116.8, 112.0 (br), 101.2; \( \nu_{\text{max}} \) / cm\(^{-1}\) 3428, 3346 (NH), 1660 (C=O); \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)) 226 (4.25), 242 (4.26), 262 (4.32), 308 (4.34), 318 (4.36), 448 (4.22). Anal. Calcd for C\(_{16}\)H\(_{11}\)N\(_3\)O: C, 73.55; H, 4.24; N, 16.08. Found: C, 72.95; H, 4.28; N, 15.88.
Reactions of 3-bromo-1-azaazulene-2-carbaldehyde (6) with o-phenylenediamine

A mixture of 6 (0.0425 g, 0.18 mmol) and o-phenylenediamine (0.0233 g, 0.22 mmol) in EtOH (10 mL) was stirred for 15 h at rt. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 2-(benzimidazol-2-yl)-3-bromo-1-azaazulene (7) (0.0233 g, 40%).

7: Red needles (from CH2Cl2-hexane), mp 150-152 °C; 1H NMR δ 13.42 (1H, br s), 8.81 (1H, d, J 10.0), 8.73 (1H, d, J 9.6), 8.20 (1H, dd, J 10.0, 9.6), 8.06 (1H, dd, J 10.0, 9.6), 8.03 (1H, dd, J 10.0, 9.6), 7.90-7.75 (1H, m), 7.70-7.55 (1H, m), 7.40-7.20 (2H, m); 13C NMR δ 155.9, 151.8, 146.7, 144.2, 140.6, 137.8, 136.3, 131.4, 130.7, 123.9, 119.7, 117.3, 114.5, 112.1, 99.0; νmax/cm−1 3423 (NH); λmax nm (log ε) 220 (4.52), 274 (4.65), 336 (4.43), 400 (4.29), 524 (3.45). Anal. Calcd for C16H10N3Br·3/4H2O: C, 56.91; H, 3.43; N, 12.44. Found: C, 57.24; H, 3.55; N, 12.31.

Reaction of ethyl 2-chloro-8-formyl-1-azaazulene-3-carboxylate (8a) with o-phenylenediamine

A mixture of 8a (0.0526 g, 0.30 mmol) and o-phenylenediamine (0.0231 g, 0.21 mmol) in EtOH (10 mL) was stirred for 4 h at rt. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave ethyl 8-(benzimidazol-2-yl)-1-azaazulene-3-carboxylate (9) (0.0190 g, 38%).

9: Orange needles (from CH2Cl2-hexane), mp 187-189 °C; 1H NMR δ 13.51 (1H, br s), 9.59 (1H, d, J 10.4), 9.52 (1H, d, J 10.0), 8.42 (1H, dd, J 10.4, 10.0), 8.24 (1H, dd, J 10.0, 10.0), 7.94-7.82 (2H, m), 7.43-7.31 (2H, m), 4.44 (2H, q, J 7.2), 1.43 (3H, t, J 7.2); 13C NMR (CDCl3) δ 163.3, 157.0, 151.9, 150.1, 148.3, 138.8, 137.1, 134.6, 134.2, 133.9, 125.2, 123.5, 120.4, 112.4, 112.2, 60.9, 14.4; νmax/cm−1 3224 (NH), 1692 (C=O), 1651 (C=N); λmax nm (log ε) 225 (4.17), 281 (4.10), 293 (4.13), 330 (3.95), 409 (4.03), 451 (3.55, sh). Anal. Calcd for C19H14N3ClO2: C, 64.87; H, 4.01; N, 11.94. Found: C, 64.73; H, 4.22; N, 12.12.

Reactions of 2-chloro-1-azaazulene-3-carbaldehyde (1a) with 2-aminophenol

a) A mixture of 1a (0.0579 g, 0.30 mmol) and 2-aminophenol (0.0331 g, 0.30 mmol) in EtOH (10 mL) was heated under reflux for 10 min. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 2-chloro-3-[(2-hydroxyphenyl)iminomethyl]-1-azaazulene (10a) (0.0512 g, 60%) and 2-[(2-hydroxyphenyl)amino]-3-[(2-hydroxyphenyl)iminomethyl]-1-azaazulene (11) (trace).

b) A mixture of 1a (0.0589 g, 0.31 mmol) and o-aminophenol (0.0356 g, 0.33 mmol) in EtOH (10 mL) was heated at 50 °C for 40 min. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 10a (0.0692 g, 80%) and 11 (trace).

c) A mixture of 1a (0.0728 g, 0.38 mmol) and 2-aminophenol (0.0582 g, 0.53 mmol) in EtOH (15 mL) was heated under reflux for 15 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 10a (trace) and 11 (0.0505 g, 37%).

10a: Orange needles (from CH2Cl2-hexane), mp 179-181 °C; 1H NMR δ 10.04 (1H, d, J 10.0), 9.11 (1H, s), 8.95 (1H, d, J 9.6), 8.30 (1H, dd, J 10.0, 9.6), 8.18 (1H, dd, J 10.0, 9.6), 8.16 (1H, dd, J
10.0, 9.6), 7.21 (1H, dd, J 7.6, 1.6), 7.10 (1H, ddd, J 8.0, 7.6, 1.6), 6.94 (1H, dd, J 8.0, 1.2), 6.88 (1H, td, J 7.6, 1.2); 13C NMR δ 158.2, 156.7, 152.7, 150.5, 142.8, 141.5, 139.5, 138.3, 137.9, 134.2, 133.6, 126.9, 119.7, 119.4, 116.1, 114.3; ν\text{max} / cm⁻¹ 3375 (OH), 1616 (C=N); λ\text{max} nm (log ɛ) 254 (4.33), 259 (4.33), 291 (4.45, sh), 298 (4.46), 324 (4.15, sh), 394 (3.97), 482 (3.25, sh). Anal. Calcd for C₁₆H₁₁N₂ClO: C, 67.97; H, 3.92; N, 9.91. Found: C, 67.87; H, 3.68; N, 9.64.

11: Orange powders (from CH₂Cl₂-hexane), mp 235-237 °C; ¹H NMR δ 11.43 (1H, s), 10.77 (1H, br s), 9.57 (1H, s), 8.88 (1H, dd, J 7.2), 8.71 (1H, d, J 10.0), 8.49 (1H, br s), 8.21 (1H, d, J 9.6), 7.78 (1H, dd, J 10.0, 9.2), 7.64 (1H, dd, J 10.0, 9.2), 7.59 (1H, d, J 7.6), 7.12 (1H, dd, J 7.6, 7.2), 7.03-6.89 (5H, m); 13C NMR δ 162.7, 159.9, 152.3, 150.5, 145.8, 145.7, 137.3, 133.3, 132.8, 131.9, 129.4, 128.2, 126.8, 119.8, 119.54, 119.47, 118.9, 115.7, 114.5, 107.6; ν\text{max} / cm⁻¹ 3415 (OH), 3259 (NH); λ\text{max} nm (log ɛ) 234 (4.22), 294 (4.41), 329 (4.40), 345 (4.40), 374 (4.39), 484 (4.14). Anal. Calcd for C₂₂H₁₇N₃O₂: C, 74.35; H, 4.82; N, 11.82. Found: C, 74.70; H, 5.05; N, 11.57.

Reactions of 2-ethoxy-1-azaazulene-3-carbaldehyde (1b) with 2-aminophenol
a) A mixture of 1b (0.0582 g, 0.29 mmol) and 2-aminophenol (0.0425 g, 0.39 mmol) in EtOH (10 mL) was heated under reflux for 24 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 2-ethoxy-3-[(2-hydroxyphenyl)iminomethyl]-1-azaazulene (10b) (0.0761 g, 90%).

b) A mixture of 1b (0.0632 g, 0.31 mmol), 2-aminophenol (0.0368 g, 0.34 mmol) and DDQ (0.0692, 0.31 mmol) in EtOH (10 mL) was heated under reflux for 24 h. The solvent was evaporated, and the residue was chromatographed with hexane-AcOEt to give a trace amount of 10b and recovered 1b (0.0440 g, 69%).

10b: Orange needles (from CH₂Cl₂-hexane), mp 205-207 °C; ¹H NMR δ 9.66 (1H, dd, J 10.0, 1.6), 8.88 (1H, s), 8.82 (1H, s), 8.41 (1H, dd, J 9.6, 1.6), 7.94-7.87 (3H, m), 7.12 (1H, dd, J 7.6, 1.6), 7.05 (1H, ddd, J 8.0, 7.6, 1.6), 6.91 (1H, dd, J 8.0, 1.6), 6.85 (1H, td, J 7.6, 1.6), 4.72 (2H, q, J 7.0), 1.48 (3H, t, J 7.0); ¹³C NMR δ 174.4, 157.0, 152.1, 150.4, 143.8, 140.1, 136.1, 133.4, 132.9, 132.6, 126.2, 119.6, 119.1, 115.8, 106.1, 65.1, 14.6; ν\text{max} / cm⁻¹ 3206 (OH); λ\text{max} nm (log ɛ) 256 (4.11), 305 (4.29), 413 (3.93). Anal. Calcd for C₁₈H₁₆N₂O₂: C, 73.96; H, 5.52; N, 9.58. Found: C, 73.77; H, 5.64; N, 9.36.

Reactions of 2-amino-1-azaazulene-3-carbaldehyde (1c) with 2-aminophenol
a) A mixture of 1c (0.0594 g, 0.35 mmol) and 2-aminophenol (0.0762 g, 0.70 mmol) in 1-BuOH (10 mL) was heated under reflux for 22 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 2-amino-3-[(2-hydroxyphenyl)iminomethyl]-1-azaazulene (10c) (0.0872 g, 91%).

b) A mixture of 1c (0.0498 g, 0.29 mmol), o-aminophenol (0.0590 g, 0.54 mmol) and I₂ (0.0386, 0.15 mmol) in 1-BuOH (10 mL) was heated under reflux for 48 h. The solvent was evaporated, and the
residue was chromatographed with hexane-AcOEt to give 10c (0.0273 g, 36%) and recovered 1b (trace).

10c: Dark red prisms (from CH2Cl2-hexane), mp 207.5-209 °C; 1H NMR δ 9.21 (1H, s), 9.01 (1H, br s), 8.50 (1H, d, J 9.9), 8.21 (2H, br s), 7.88 (1H, d, J 9.8), 7.64 (1H, dd, J 9.9, 9.8), 7.57 (1H, dd, J 9.9, 9.8), 7.45 (1H, dd, J 9.9, 9.8), 7.32 (1H, dd, J 7.9, 1.4), 7.04 (1H, ddd, J 8.0, 7.9, 1.4), 6.92-6.82 (2H, m); 13C NMR (CDCl3) δ 168.3, 161.2, 153.1, 150.7, 148.0, 139.2, 133.9, 132.6, 132.2, 129.2, 127.7, 125.4, 121.0, 117.6, 115.3, 106.6; νmax / cm⁻¹ 3387 (OH), 3284, 3235 (NH); λmax nm (log ε) 241 (4.69), 286 (4.54), 349 (4.44), 362 (4.38, sh), 461 (4.13).

Reactions of 2-oxo-1,2-dihydro-1-azaazulene-3-carbaldehyde (4) with 2-aminophenol
A mixture of 4 (0.0650 g, 0.38 mmol) and 2-aminophenol (0.0615 g, 0.56 mmol) in EtOH (10 mL) was heated under reflux for 12 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 3-[(2-hydroxyphenyl)iminomethyl]-1-azaazulen-2(1H)-one (12) (0.0531 g, 54%).

12: Orange powders (from CH2Cl2-hexane), mp 247-250 °C (decomp); 1H NMR δ 9.19 (1H, d, J 10.8), 8.67 (1H, t, J 10.0), 7.59 (1H, d, J 10.8, 10.0), 7.46 (1H, d, J 9.6), 7.31 (1H, dd, J 10.0, 9.6), 7.07 (1H, d, J 7.6), 7.03 (1H, ddd, J 8.0, 7.6, 1.2), 6.89 (1H, dd, J 8.0, 1.2), 6.83 (1H, td, J 7.6, 1.2), (NH and OH protons were not observed); 13C NMR δ 170.1, 152.9, 150.4, 148.1, 142.5, 139.9, 136.0, 135.3, 131.5, 130.0, 126.2, 119.6, 118.8, 117.8, 115.8, 107.6; νmax / cm⁻¹ 3407 (NH, OH), 1654 (C=O); λmax nm (log ε) 258 (4.16), 287 (4.14), 363 (3.41), 422 (3.76, sh), 444 (3.92), 475 (3.63, sh), 510 (3.40, sh). Anal. Calcd for C16H12N2O2: C, 72.72; H, 4.58; N, 10.64. Found: C, 72.83; H, 4.82; N, 9.89.

Reactions of 2-ethoxy-1-azaazulene-3-carbaldehyde (1b) with 2-aminothiophenol
A mixture of 1b (0.0816 g, 0.41 mmol) and 2-aminothiophenol (0.0538 g, 0.43 mmol) in EtOH (10 mL) was heated under reflux for 4.5 h. The mixture was evaporated, and the chromatography of the residue with hexane-AcOEt gave 3-(benzothiazol-2-yl)-2-ethoxy-1-azaazulene (13b) (0.0823 g, 66%).

13b: Orange needles (from CH2Cl2-hexane), mp 177-179 °C; 1H NMR δ 9.94 (1H, d, J 10.0), 8.53-8.44 (1H, m), 8.14 (1H, d, J 7.6), 8.09 (1H, d, J 8.0), 8.08-8.01 (1H, m), 8.01-7.93 (2H, m), 7.54 (1H, dd, J 8.0, 7.6), 7.42 (1H, t, J 7.6), 4.85 (2H, q, J 7.0), 1.59 (3H, t, J = 7.0); 13C NMR δ 171.9, 159.8, 156.1, 152.8, 143.4, 136.5, 133.5, 133.3, 133.1, 133.0, 126.2, 124.3, 121.7, 121.6, 102.6, 66.0, 14.8; νmax / cm⁻¹ 1600 (C=N); λmax nm (log ε) 222 (4.52), 258 (4.37), 270 (4.32, sh), 315 (4.52, sh), 326 (4.66), 396 (4.12), 464 (3.53, sh). Anal. Calcd for C18H14N2OS: C, 70.56; H, 4.61; N, 9.14. Found: C, 70.89; H, 4.70; N, 9.05.

Reactions of 2-amino-1-azaazulene-3-carbaldehyde (1c) with 2-aminothiophenol
a) A mixture of 2-amino-1-azaazulene-3-carbaldehyde (1c) (0.0427 g, 0.25 mmol) and 2-aminothiophenol (0.103 g, 0.82 mmol) in 1-BuOH (30 mL) was heated at 125 °C for 190 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt to give
2-amino-3-(2-benzothiazoyl)-1-azaazulene (13c) (0.0246 g, 36%), 2-amino-1-azaazulene (14) (0.0212 g, 59%), and a trace amount of benzothiazole.

b) A mixture of 1c (0.0357 g, 0.21 mmol), 2-aminothiophenol (0.0332 g, 0.27 mmol), and I₂ (0.0262 g, 0.10 mmol) in 1-BuOH (25 mL) was refluxed for 24 h. To the mixture 10% Na₂S₂O₃ solution (18 mL) was added, and the precipitate was filtered off. The filtrate was evaporated, and the chromatography of the residue with hexane-AcOEt gave 13c (0.0209 g, 36%), 2-amino-3-(2-aminophenylmercaptocalbonyl)-1-azaazulene (15) (0.0102 g, 17%), and 1c (0.0026 g, 7%).

c) A mixture of 1c (0.0348 g, 0.20 mmol), 2-aminothiophenol (0.0374 g, 0.30 mmol), and sat. FeCl₃ solution (2.0 mL) in 1-BuOH (10 mL) was refluxed for 24 h. To the mixture water (30 ml) was added, and the mixture was extracted with CHCl₃. The extract was dried over Na₂SO₄, and the solvent was evaporated. Chromatography of the residue with hexane-AcOEt gave 13c (0.0176 g, 31%) and 1c (0.0028 g, 11%).

13c: Orange prisms (from EtOAc), mp 247-250 °C; ¹H NMR δ 8.49 (1H, d, J 10.1) 8.48 (2H, s), 8.15 (1H, dd, J 9.8), 7.81 (1H, dd, J 10.1, 9.8), 7.79 (1H, dd, J 10.0, 9.9), 7.59 (1H, dd, J 9.0, 10.6), 7.42 (1H, d, J 8.0, 0.32), 7.33 (1H, d, J 7.9, 1.0), 7.23 (1H, dd, J 7.9, 1.0), 7.18 (1H, d, J 7.9, 1.0), 6.95 (1H, d, J 7.9, 1.0), 6.69 (1H, d, J 7.9, 1.0), 6.56 (1H, d, J 7.9, 1.0), 5.67 (2H, br s), 4.22 (2H, br s); ¹³C NMR (CDCl₃) δ 161.17, 158.94, 151.67, 133.62, 132.18, 131.99, 131.13, 127.51, 126.56, 125.04, 124.34, 122.52, 121.69, 121.45, 120.37, 109.04; ν max / cm⁻¹ 3342, 3276 (NH); λ max nm (log ε) 222 (4.39), 260 (4.23), 294 (4.13), 338 (4.55), 460 (4.04). Anal. Calcd. for C₁₆H₁₁N₃S: C, 69.29; H, 4.00; N, 15.15. Found: C, 69.69; H, 4.14; N, 14.88.

15: Red plates (from CHCl₃-hexane), mp 190-191 °C; ¹H NMR (CDCl₃) δ 8.21 (1H, d, J 10.2), 7.98 (1H, d, J 9.7), 7.57 (1H, dd, J 10.0, 9.7), 7.51 (1H, dd, J 10.2, 9.5), 7.42 (1H, d, J 10.0, 9.5), 7.01 (1H, d, J 7.9, 1.1), 6.92 (1H, dd, J 7.8, 1.4), 6.71 (1H, d, J 7.9, 1.1), 6.59 (1H, d, J 7.8, 1.2), 5.69 (2H, br s), 4.22 (2H, br s); ¹³C NMR (CDCl₃) δ 196.61, 168.67, 159.16, 149.60, 145.34, 131.63, 131.45, 130.79, 130.79, 128.11, 127.31, 127.25, 119.64, 119.58, 115.83, 97.07; ν max / cm⁻¹ 3430, 3343, 3277, 3214 (NH); λ max nm (log ε) 239 (4.29, sh), 280 (4.47), 320 (4.27, sh), 434 (3.61). Anal. Calcd for C₁₆H₁₃N₃OS: C, 65.06; H, 4.44; N, 14.23. Found: C, 65.28; H, 4.79; N, 14.10.

Reactions of 2-oxo-1,2-dihydro-1-azaazulen-3-carbaldehyde (4) with 2-aminothiophenol
A mixture of 4 (0.0538 g, 0.31 mmol) and 2-aminothiophenol (0.0404 g, 0.32 mmol) in EtOH (10 mL) was heated under reflux for 15 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 3-(2-benzothiazoyl)-1-azaazulen-2(1H)-one (16) (0.0460 g, 53%).

16: Red plates (from EtOH), mp >300 °C; ¹H NMR δ 12.38 (1H, s), 9.51 (1H, d, J 11.2), 8.10 (1H, d, J 7.6), 8.04 (1H, d, J 8.0), 7.77 (1H, dd, J 11.2, 10.8), 7.67 (1H, dd, J 10.0, 9.6), 7.58 (1H, d, J 9.6), 7.52 (1H, dd, J 10.8, 10.0), 7.39 (1H, dd, J 7.6, 7.2); ¹³C NMR δ 167.5, 160.2, 152.4, 146.2, 141.0, 136.1, 135.4, 133.4, 132.1, 129.7, 125.9, 124.1, 121.6, 121.4, 118.2, 104.3; ν max / cm⁻¹ 3436 (NH), 1684 (C=O);
Reactions of 3-bromo-1-azaazulene-2-carbaldehyde (6) with 2-aminothiophenol

A mixture of 6 (0.0434 g, 0.18 mmol) and 2-aminothiophenol (0.0262 g, 0.21 mmol) in EtOH (10 mL) was heated under reflux for 1 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 2-(benzothiazol-2-yl)-3-bromo-1-azaazulene (17) (0.0198 g, 32%).

17: Reddish violet needles (from CH2Cl2-hexane); mp 119-121 °C. 1H NMR δ 8.88 (1H, d, J 9.6), 8.79 (1H, d, J 9.6), 7.63 (1H, ddd, J 8.0, 7.2, 1.2), 7.57 (1H, ddd, J 8.0, 7.2, 0.8); 13C NMR δ 163.0, 155.8, 154.3, 144.5, 139.2, 137.7, 135.4, 131.8, 131.1, 126.8, 126.4, 123.7, 122.4, 98.4; νmax / cm⁻¹ 1611 (C=N); λmax nm (log ε) 223 (4.22), 242 (3.91), 276 (4.12), 302 (4.04), 331 (4.19), 379 (3.98), 397 (3.97), 533 (3.11).  Anal. Calcd for C16H9N2BrS: C, 56.32; H, 2.66; N, 8.21. Found: C, 56.67; H, 2.89; N, 8.10.

Reaction of ethyl 2-ethoxy-8-formyl-1-azaazulene-3-carboxylate (8b) with 2-aminothiophenol

A mixture of 8b (0.0329 g, 0.12 mmol) and 2-aminothiophenol (0.0229 g, 0.18 mmol) in EtOH (10 mL) was stirred for 15 min at rt. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave ethyl 8-(2-benzothiazolyl)-1-azaazulene-3-carboxylate (18) (0.0244 g, 54%).

18: Yellow needles (from CH2Cl2-hexane), mp 176-178 °C; 1H NMR (CDCl3) δ 9.21 (1H, dd, J 9.6, 1.6), 8.34 (1H, dd, J 9.6, 1.2), 7.70 (1H, td, J 9.6, 1.2), 7.63 (1H, ddd, J 9.6, 9.6, 1.6), 7.02 (1H, dd, J 8.0, 1.2), 6.98 (1H, ddd, J 8.0, 7.6, 1.2), 6.80 (1H, dd, J 8.0, 1.2), 6.76 (1H, ddd, J 8.0, 7.6, 1.2), 4.83 (2H, q, J 7.0), 4.43 (2H, q, J 7.2), 1.56 (3H, t, J 7.2), 1.44 (3H, t, J 7.0); 13C NMR (CDCl3) δ 173.6, 165.1, 164.2, 152.5, 151.6, 149.9, 139.2, 134.0, 133.5, 133.4, 132.8, 126.2, 125.7, 123.7, 121.4, 100.7, 67.3, 60.1, 14.7, 14.5; νmax / cm⁻¹ 1687 (C=O); λmax nm (log ε) 226 (4.62), 246 (4.35, sh), 299 (4.47), 343 (4.34, sh), 351 (4.36), 351 (4.36), 375 (4.38), 397 (4.33), 465 (3.50).  Anal. Calcd for C21H18N2O3S: C, 66.65; H, 4.79; N, 12.68. Found: C, 66.88; H, 4.79; N, 12.52.

Reactions of 2-chloro-1-azaazulene-3-carbaldehyde (1a) with 2-aminothiophenol

A mixture of 1a (0.0632 g, 0.33 mmol) and 2-aminothiophenol (0.0469 g, 0.38 mmol) in 1-BuOH (5 mL) was heated under reflux for 45 h. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 5-thia-6,13-diazacyclohept[a]benz[f]azulene (20) (0.0357 g, 41%).

20: Reddish violet powders (from CH2Cl2-hexane), mp 251-253 °C; 1H NMR δ 10.61 (1H, s), 7.41 (1H, d, J 8.8), 7.21-7.02 (4H, m), 7.06 (1H, dd, J 7.6, 1.2), 7.04 (1H, ddd, J 8.0, 7.6, 1.2), 6.89 (1H, dd, J 8.0, 1.2), 6.84 (1H, J 7.6, 1.2); 13C NMR δ 158.6, 136.5, 130.8, 130.2, 128.7, 127.2, 126.7, 125.2, 123.0, 117.5, 115.5, 97.8; νmax / cm⁻¹ 1630 (C=N); λmax nm (log ε) 251 (4.18), 274 (4.32), 312 (3.93), 342 (4.15), 363 (4.06, sh), 454 (3.43, sh), 479 (3.55), 509 (3.46, sh), 543 (3.40, sh), 602 (3.11, sh), 653 (2.65, sh).
Reactions of 2-chloro-1-azaazulene-3-carbaldehyde (1a) with 4-amino-3-mercapto-4H-1,2,4-triazole

A mixture of 1a (0.0597 g, 0.31 mmol) and 4-amino-3-mercapto-1,2,4-triazole (21a) (0.0415 g, 0.36 mmol) in 1-BuOH (10 mL) was heated under reflux for 3 min. The solvent was evaporated, and the chromatography of the residue with hexane-AcOEt gave 4-thia-2,3,5,12,12a-pentaazaazuleno[5,6-b]-azulene (22a) (0.0391 g, 49%).

22a: Orange powders (from CHCl₃-EtOH), mp 202-203 °C; ¹H NMR (TFA-d) δ 9.82 (1H, s), 9.52 (1H, d, J 9.6), 9.40 (1H, d, J 9.6), 9.33 (1H, s), 9.02 (1H, t, J 9.6), 8.87 (2H, t, J 9.6); ¹³C NMR (TFA-d) δ 156.3, 152.3, 151.0, 148.8, 147.8, 146.2, 144.8, 144.1, 143.9, 143.6, 139.5, 119.5; ν max / cm⁻¹ 1614 (C=N); λ max nm (log ε) 233 (3.68), 290 (4.31, sh), 315 (3.78, sh), 462 (3.40). Anal. Calcd for C₁₂H₇N₅S: C, 56.90; H, 2.79; N, 27.65. Found: C, 57.02; H, 3.14; N, 26.93.

In a similar manner, reactions of 1a with 21b, 21c, and 21d gave 22b, 22c and 22d in 67%, 33%, and 43%, respectively.

22b: Orange powders (from EtOH-CHCl₃), mp 270-272 °C; ¹H NMR (TFA-d) δ 9.50 (1H, d, J 9.6), 9.36 (1H, d, J 10.0), 9.32 (1H, s), 9.01 (1H, dd, J 10.0, 9.6), 8.86 (1H, dd, J 10.0, 9.6), 8.84 (1H, dd, J 10.0, 9.6), 2.99 (3H, s); ¹³C NMR (TFA-d) δ 159.2, 155.4, 152.3, 151.0, 148.9, 146.0, 144.9, 144.1, 143.8, 143.7, 139.4, 119.4, 11.0; ν max / cm⁻¹ 1615 (C=N); λ max nm (log ε) 239 (4.20), 275 (4.42), 318 (4.59), 364 (3.90, sh), 465 (3.27). Anal. Calcd for C₁₃H₉N₅S: C, 58.41; H, 3.39; N, 26.20. Found: C, 58.43; H, 3.40; N, 26.93.

22c: Yellow prisms (from EtOH-CHCl₃), mp 224-226 °C; ¹H NMR (TFA-d) δ 9.47 (1H, d, J 10.0), 9.32 (1H, d, J 10.0), 9.24 (1H, s), 9.00 (1H, t, J 10.0), 8.84 (2H, t, J 10.0); ¹³C NMR (TFA-d) δ 154.0, 152.0, 151.0, 149.4, 149.0 (q, J_CCF 45.0), 146.9, 144.7, 143.9, 143.6, 143.5, 139.1, 119.9, 118.5 (q, J_CF 271.7); ν max / cm⁻¹ 1617 (C=N); λ max nm (log ε) 253 (3.86), 270 (4.01), 275 (4.42), 314 (4.59), 364 (3.53, sh), 456 (2.99). Anal. Calcd for C₁₆H₁₂N₄: C, 48.60; H, 1.88; N, 21.80. Found: C, 49.10; H, 1.95; N, 21.55.

22d: Yellow prisms (from EtOH-CHCl₃), mp 249-250 °C; ¹H NMR (TFA-d) δ 9.57-9.50 (1H, m), 9.40-9.35 (2H, m), 9.24-9.16 (2H, m), 9.05-8.96 (3H, m), 8.89-8.81 (2H, m); ¹³C NMR (TFA-d) δ 154.7, 152.7, 152.1, 151.1, 149.6, 146.9, 144.7, 144.6, 143.9, 143.8, 143.6, 139.2, 129.4, 119.3; ν max / cm⁻¹ 1633 (C=N); λ max nm (log ε) 240 (3.74), 272 (3.99, sh), 288 (4.27), 322 (4.45), 370 (3.72, sh), 458 (3.14). Anal. Calcd for C₁₇H₁₀N₆S: C, 61.80; H, 3.05; N, 25.44. Found: C, 61.54; H, 3.01; N, 25.78.

X-Ray structure determination

Crystal data of 3c: brown prismatic, C₁₇H₁₆N₄Cl, M = 347.25, monoclinic, space group P2₁/n, a =
\[ a = 6.125(6) \text{ Å}, \quad b = 8.883(8) \text{ Å}, \quad c = 23.697(5) \text{ Å}, \quad \beta = 94.82(5)^\circ, \quad V = 1284.8(17) \text{ Å}^3, \quad Z = 8, \quad D_{\text{calc}} = 1.774 \text{ g/cm}^3, \]
crystal dimensions 0.12 × 0.10 × 0.08 mm. Data were measured on a Rigaku RAXIS-RAPID radiation diffractometer with graphite monochromated CuK\(\alpha\) radiation. Total 15184 reflections (2194 unique) were collected using \(\omega-2\theta\) scan technique with a \(2\theta\) range of 136.4\(^\circ\). The structure was solved by direct methods (SHELX97)\(^{22}\) and expanded using Fourier techniques\(^{23}\), and refined a full-matrix least squares methods with 200 variables and 2194 observed reflections \([I > 2\sigma(I)]\). The final refinement converged to \(R = 0.0304\) and \(R_w = 0.0844\). All calculations were performed using the CrystalStructure crystallographic software package.\(^{20,21}\)

**Crystal data of 22c:**

Yellow block, \(C_{13}H_{6}F_{3}N_{5}S, \ M = 321.28\), orthorhombic, space group Pbca, \(a = 13.32671(13) \text{ Å}, \quad b = 7.37808(13) \text{ Å}, \quad c = 24.4650(7) \text{ Å}, \quad V = 2405.53(8) \text{ Å}^3, \quad Z = 8, \quad D_{\text{calc}} = 1.774 \text{ g/cm}^3, \)
crystal dimensions 0.12 × 0.10 × 0.08 mm. Data were measured on a Rigaku RAXIS-RAPID radiation diffractometer with graphite monochromated CuK\(\alpha\) radiation. Total 15184 reflections (2194 unique) were collected using \(\omega-2\theta\) scan technique with a \(2\theta\) range of 136.4\(^\circ\). The structure was solved by direct methods (SHELX97)\(^{22}\) and expanded using Fourier techniques\(^{23}\), and refined a full-matrix least squares methods with 200 variables and 2194 observed reflections \([I > 2\sigma(I)]\). The final refinement converged to \(R = 0.0304\) and \(R_w = 0.0844\). All calculations were performed using the CrystalStructure crystallographic software package.\(^{20,21}\)

**Biological assay**

HeLa S3 cells were obtained from AIST and used after cultivation. The cultivated HeLa S3 cells were cell counted and the culture fluid was prepared to the cell consistency of \(2 \times 10^4\) cells/mL. The compounds added to the medium in DMSO solutions. To the aliquot of the culture fluid, which was incubated for 3 h at 37 °C, the test sample was added and then the culture fluid was incubated for 72 h. To the culture fluid, MTT (3-[4,5-dimethylthiazol]-2-yl-2,5-diphenyltetrazolium bromide) solution was added, and incubated for 4 h. Then the sample was centrifuged at 3000 rpm for 10 min at 4 °C, and the solvent was evaporated. Then DMSO was added to the obtained mixture. The MTT-formazan was
dissolved by plate-mixing and OD540 was measured. The rate of outlive determined to refer with un-dosed control. Dose-response curve was drawn up and IC$_{50}$ was pursued. Every experiment in the cytotoxic assay was replicated twice in order to define the IC values.

REFERENCES AND NOTE